

EFFECT OF BOTANICAL EXTRACTS ON SOIL POPULATIONS OF *FUSARIUM* AND OTHER SOILBORNE PATHOGENS

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Fusarium wilt of chrysanthemum (*Chrysanthemum morifolium* Ramat.) caused by *Fusarium oxysporum* f. sp. *chrysanthemi*, (F.o.c.) is one of the most widespread and destructive diseases of this major horticultural crop. Initial symptoms appear as chlorosis and distortion of the lower leaves, often on one side of the plant. Chlorotic wedges, necrosis, and plant stunting become more pronounced as the disease progresses. Wilting occurs on the affected side of the plant, followed by vascular discoloration and stem necrosis. The entire plant wilts and dies as the pathogen moves into the stem. Presently, preplant soil fumigation with methyl bromide is being used to control the disease. However, the U.S. Environmental Protection Agency set January 1, 2001, as the phaseout date for methyl bromide in the United States. Since the Clean Air Act allows no exceptions, all uses of methyl bromide will be banned.

The long-term goal of the research in our laboratory in response to the EPA action is to develop and evaluate new or existing alternative control methods for soilborne plant pathogens to replace methyl bromide in ornamental crop production systems. Biologically-based and environmentally-safe alternatives, such as biological control agents, natural plant products, and cultural methods, are being investigated for possible use in integrated management strategies.

The objective of the present research is to evaluate several formulated plant extracts for their effectiveness in reducing soil populations of *Fusarium oxysporum* f. sp. *chrysanthemi*. We infested soil with F.o.c., treated the soil with dilutions of the formulated products under standard conditions and assayed the population density of F.o.c. over time. If natural plant products can reduce populations of soilborne plant pathogens, then these plant extracts have potential as environmentally-safe alternatives for methyl bromide as critical components in integrated management programs.

Greenhouse soil was infested with 1.1×10^6 spores of F.o.c./cm³ of soil and 150 cm³ aliquots were distributed into sterile 400-ml beakers. Uninfested soil was used as a control. Soil was treated by incorporating 5.0 ml of the various extracts into each of three beakers (replications) per treatment. One, 5, and 10% aqueous emulsions of formulated extracts were evaluated in separate experiments. Ban-rot, a standard fungicide, was applied at low, medium, and high labeled rates (4, 8, and 12 oz per 100 gal, respectively). Experimental treatments included 1) control soil - no F.o.c., 2) F.o.c. only, and F.o.c. with 3) Clove (70% formulated clove oil), 4) Neem (90% formulated neem oil), 5) Pepper (formulated chili extract and essential oil of mustard), 6) Banrot fungicide, and 7) Cassia (formulated extract of cassia tree). Population densities of F.o.c. were determined using dilution plate techniques at 0 (before soil treatment), 1, 3, 7, 14, and 21 days after soil treatment.

Soil treated with 1% aqueous emulsions of the botanical extracts or the Banrot fungicide did not significantly reduce the soil population of F.o.c. compared to the untreated control soil (F.o.c. only). However, treatment of soil with 5 and 10% aqueous emulsions

of the botanical extracts resulted in significant differences among treatment mean values at each assay date.

The formulated clove and cassia extracts significantly reduced the population density of F.o.c. compared to the untreated control soil only when added to soil as 10% aqueous emulsions. The formulated pepper extract significantly reduced the population density of F.o.c. compared to the untreated control soil when added to soil as either a 5 or 10% aqueous emulsion. The reduction in F.o.c. population density also was significantly greater than that achieved with the clove or cassia extracts. When the formulated pepper extract was added to soil as a 5 or 10% aqueous emulsion, the population density of F.o.c. was reduced to low levels, but increased over time.

The treatment of the soil with a formulated neem extract increased the population density of F.o.c. when added to soil as both a 5 and 10% aqueous emulsion. Treatment of the soil with the fungicide Banrot generally did not affect the population density of F.o.c. in any experiment at any tested rate.

The data does not support the null hypothesis of equal treatment means when the formulated plant extracts were added to soil as 5 or 10% aqueous emulsions. Significant differences among treatment means were observed. The pepper, clove and cassia extracts reduced population densities of F.o.c. in soil by 99.9, 97.5, and 96.1%, respectively, after 3 days incubation when added as 10% aqueous emulsions. When added as a 5% aqueous emulsion, the pepper extract also reduced population densities of F.o.c. by 99.9%.

Soil populations of F.o.c. were lowest after 3-7 days of incubation when the soil was treated with aqueous emulsions of the formulated pepper extract. However, populations of F.o.c. increased over time in these treatments. One explanation may be that the extract breaks down rapidly in soil. Observation of the lack of background microflora on dilution plates suggests that the pepper extract may effect a wide range of soil fungi, and may create a biological vacuum. This was not observed with the other extracts. The small surviving population of F.o.c. which has been shown to rapidly colonize fumigated soil in the absence of competition, may have flourished in the soil environment once the extract was no longer present and the natural microflora at low population levels. This also may be true for the clove and cassia extracts, but to a lesser extent. Further research on the soil ecology when these extracts are added to soil is needed.

The observed reduction in the pathogen population coupled with an environmentally friendly rapid breakdown in soil suggests that these extracts may have an important role in biologically-based management strategies. One plausible scenario is that a natural extract be incorporated into soil to initially reduced the pathogen population. This would be followed 3-10 days later by application of a biological agent compatible with the extract to rapidly colonize the treated soil to suppress further development of the pathogen, thus achieving disease control. Experiments in the greenhouse are in progress to test these hypotheses and to determine how the extracts effect seedling emergence and plant growth.

Soil population densities of *Fusarium oxysporum f.sp. chrysanthemi* (F.o.c.) over time as affected by soil treatment with botanical extracts and a standard fungicide.

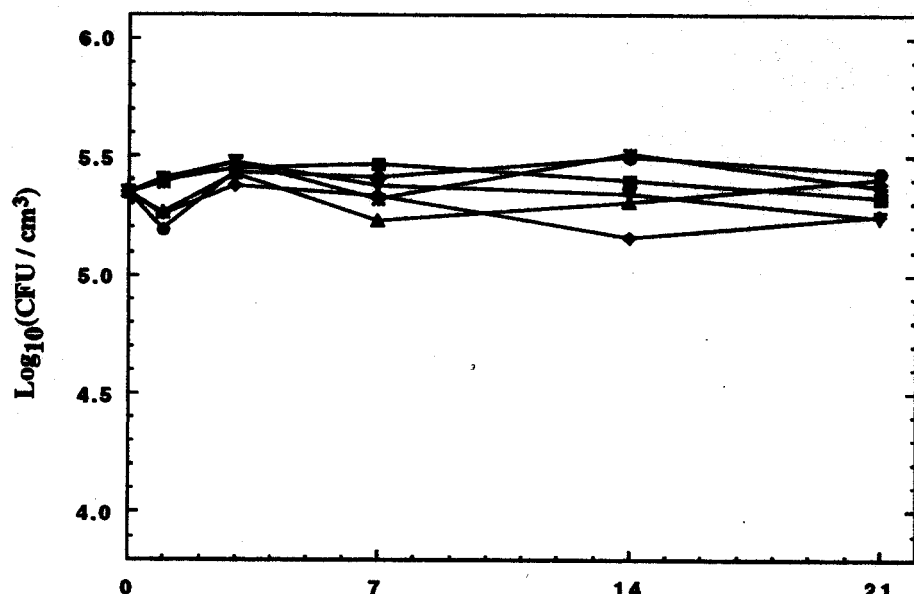
Treatment		Days After Treatment									
(1 %)		1		3		7		14		21	
F.o.c	1	5.40	a	5.45	a	5.48	a	5.40	a	5.32	a
Clove		5.25	a	5.38	a	5.33	a	5.15	a	5.25	a
Neem		5.19	a	5.43	a	5.41	a	5.49	a	5.43	a
Pepper		5.26	a	5.43	a	5.22	a	5.30	a	5.40	a
Banrot low)		5.40	a	5.47	a	5.38	a	5.34	a	5.24	a
Cassia	1	5.39	a	5.48	a	5.32	a	5.51	a	5.36	a

Treatment		Days After Treatment									
(5%)		1		3		7		14		21	
F.o.c		5.40	a	5.45	ab	5.48	ab	5.40	bc	5.32	b
Clove		5.14	a	5.14	b	5.28	b	5.40	bc	5.36	b
Neem		5.65	a	5.91	a	5.98	a	5.97	a	5.99	a
Pepper		5.02	a	1.91	c	1.85	c	3.59	d	4.49	c
Banrot(med)		5.49	a	5.60	ab	5.33	b	5.17	c	5.18	b
Cassia		4.95	a	5.26	b	5.34	b	5.58	ab	5.26	b

Treatment		Days After Treatment									
(10%)		1		3		7		14		21	
F.o.c		5.40	a	5.45	ab	5.48	a	5.40	ab	5.32	b
Clove		3.78	b	3.85	c	4.10	b	4.48	c	4.50	c
Neem		5.51	a	5.87	a	5.98	a	5.87	a	5.94	a
Pepper		3.32	b	1.31	d	1.55	c	2.00	d	3.02	e
Banrot (hi)		5.59	a	5.41	b	5.34	a	5.19	b	5.17	b
Cassia		3.95	b	4.04	c	3.48	b	4.35	c	3.93	d

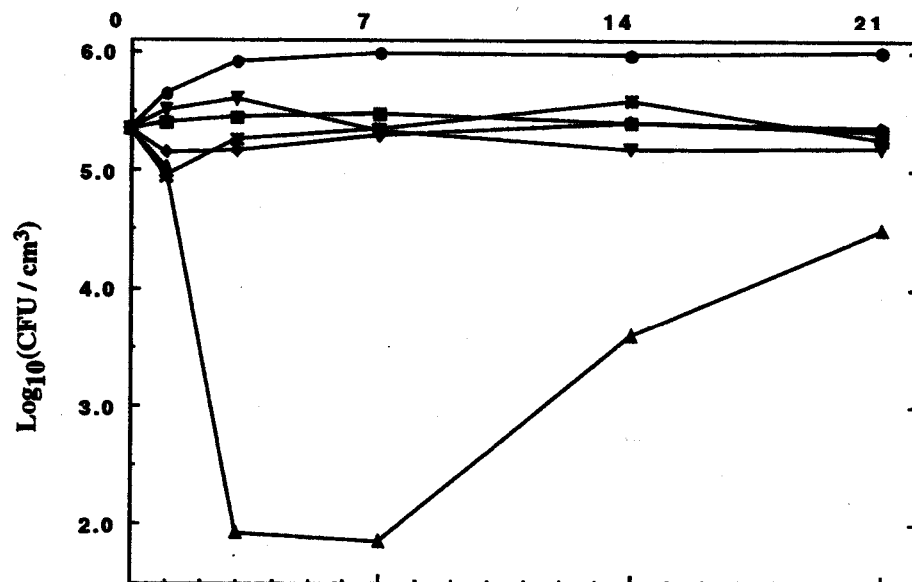
Mean values are log10 transformation of colony forming units (CFU) per cubic centimeter (cm³) of soil. Data represent the average of 2-6 experiments with three replications per treatment per experiment.

For each day in each table, mean values in the same column followed by the same letter are not significantly different at P = 0.05 based on Fisher's protected least significant difference.



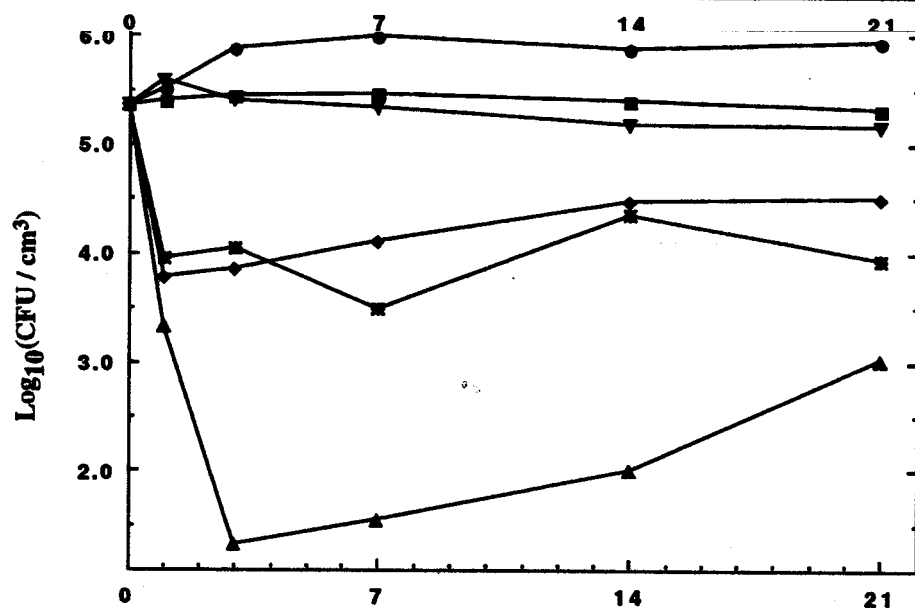
1%

- F.o.c.
- ◆— Clove
- Neem
- ▲— Pepper
- ▼— Banrot low
- *— Cassia



5%

- F.o.c.
- ◆— Clove
- Neem
- ▲— Pepper
- ▼— Banrot med
- *— Cassia



10%

- F.o.c.
- ◆— Clove
- Neem
- ▲— Pepper
- ▼— Banrot high
- *— Cassia

Days After Treatment

Plots of \log_{10} transformation of Colony forming units (CFU) per cubic centimeter (CM^3) of son over time for the effect of soil treatment with botanical extracts and a standard fungicide on the population density of *Fusarium oxysporum f.sp. chrysanthemi* in soil. Each point represents the mean of 2-6 experiments with three replications per treatment experiment